**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**
**REGION VIII, MONTANA OFFICE**
**FEDERAL BUILDING, 301 S. PARK, DRAWER 10096**
**HELENA, MONTANA 59626-0096**

Ref: 8MO

April 28, 1997

Mr. Ken Meckel, Team Leader
Tally Lake Ranger District
1335 Highway 93 West
Whitefish, MT 59937

Re: Tansy Ragwort Control Project
Draft Environmental Impact
Statement

Dear Mr. Meckel:

In accordance with our responsibilities under the National Environmental Policy Act (NEPA) and Section 309 of the Clean Air Act, the Environmental Protection Agency, Region VIII, Montana Office (EPA) reviewed the above-referenced Draft Environmental Impact Statement (DEIS).

The EPA supports the Forest Service's goal of controlling the tansy ragwort weed infestation in the Tally Lake Ranger District. However, we have concerns regarding potential effects of herbicide spraying on surface and ground water quality and wetland functions. We are particularly concerned since it is likely that the area to be sprayed may be wetter than normal due to higher than average precipitation this winter. Such conditions exacerbate the potential for herbicide drift into wet areas.

We are pleased that the District Ranger's March 25, 1997 letter transmitting the DEIS indicated that hand spraying of clopyralid, rather than aerial spraying, would be incorporated into the preferred alternative in the spring of 1997. We believe improved control over location to be sprayed, that is available with hand spraying, will reduce potential herbicide drift to streams and wetlands during the wet spring 1997 period.

We are also concerned about the lack of adequate information in the DEIS regarding ground water depths, soil types, wetlands, and the water quality/aquatics monitoring program. Additional information is needed to fully assess and mitigate all potential impacts of the proposed management actions.

Our more detailed comments, questions, and concerns regarding the analysis, documentation, and/or potential environmental impacts of the Tansy Ragwort Control Project are enclosed for your review and consideration as you complete the Final Environmental Impact Statement (FEIS).

Based on the procedures EPA uses to evaluate the adequacy of the information and the potential environmental impacts of the proposed action and alternatives in an EIS, the Tansy Ragwort Control Project DEIS has been rated as Category EC-2 (Environmental Concerns - Insufficient Information). A copy of EPA's rating criteria is attached.

The EPA appreciates the opportunity to review and comment on the DEIS. If we may provide further explanation of our concerns please contact Mr. Steve Potts of my staff in Helena at (406) 441-1140 ext. 232.

Sincerely,

John F. Wardell
Director
Montana Office

Enclosure

cc: Carol Campbell/Virginia Rose, EPA, 8EPR-EP, Denver
Mike Hammer, EPA, 8EPR-EP, Denver
Ed Stearns, EPA, 8EPR-EP, Denver
Ann Puffer, Forest Service-Region 1, EAPS, Missoula
Jim Olivarez, Forest Service Region 1, FRM, Missoula
Donna Rise, Montana Dept. of Agriculture, Helena
Elaine Suriano, EPA, OFA, Mailcode 2252A, Washington DC

## SUMMARY OF RATING DEFINITIONS

### ENVIRONMENTAL IMPACT OF THE ACTION

#### LO--LACK OF OBJECTIONS

The EPA review has not identified any potential environmental impacts requiring substantive changes to the proposal. The review may have disclosed opportunities for application of mitigation measures that could be accomplished with no more than minor changes to the proposal.

#### EC--ENVIRONMENTAL CONCERNS

The EPA review has identified environmental impacts that should be avoided in order to fully protect the environment. Corrective measures may require changes to the preferred alternative or application of mitigation measures that can reduce the environmental impact. EPA would like to work with the lead agency to reduce these impacts.

#### EO--ENVIRONMENTAL OBJECTIONS

The EPA review has identified significant environmental impacts that must be avoided in order to provide adequate protection for the environment. Corrective measures may require substantial changes to the preferred alternative or consideration of some other project alternative (including the no action alternative or a new alternative). EPA intends to work with the lead agency to reduce these impacts.

#### EU--ENVIRONMENTALLY UNSATISFACTORY

The EPA review has identified adverse environmental impacts that are of sufficient magnitude that they are unsatisfactory from the standpoint of public health or welfare or environmental quality. EPA intends to work with the lead agency to reduce these impacts. If the potentially unsatisfactory impacts are not corrected at the final EIS stage, this proposal will be recommended for referral to the CEQ.

### ADEQUACY OF THE IMPACT STATEMENT

#### CATEGORY 1--ADEQUATE

EPA believes the draft EIS adequately sets forth the environmental impact(s) of the preferred alternative and those of the alternatives reasonably available to the project or action. No further analysis or data collection is necessary, but the reviewer may suggest the addition of clarifying language or information.

#### CATEGORY 2--INSUFFICIENT INFORMATION

The draft EIS does not contain sufficient information for EPA to fully assess environmental impacts that should be avoided in order to fully protect the environment, or the EPA reviewer has identified new reasonably available alternatives that are within the spectrum of alternatives analyzed in the draft EIS, which could reduce the environmental impacts of the action. The identified additional information, data, analyses, or discussion should be included in the final EIS.

#### CATEGORY 3--INADEQUATE

EPA does not believe that the draft EIS adequately assesses potentially significant environmental impacts of the action, or the EPA reviewer has identified new, reasonably available alternatives that are outside of the spectrum of alternatives analyzed in the draft EIS, which should be analyzed in order to reduce the potentially significant environmental impacts. EPA believes that the identified additional information,data, analyses, or discussions are of such magnitude that they should have full public review at a draft stage. EPA does not believe that the draft EIS is adequate for the purposes of the NEPA and/or Section 309 review, and thus should be formally revised and made available for public comment in a supplemental or revised draft EIS. On the basis of the potential significant impacts involved, this proposal could be a candidate for referral to the CEQ.

*From: EPA Manual 1640, "Policy and Procedures for the Review of Federal Impacting the Environment."

**EPA Comments on Tansy Ragwort Control Project Draft Environmental Impact Statement**

**Tally Lake Ranger District, Flathead National Forest**

BRIEF PROJECT OVERVIEW:

The Flathead National Forest, Tally Lake Ranger District, has evaluated no action, and three action alternatives to address an approximately 1,000 acre infestation of tansy ragwort (Senecio jacobaea L.). The tansy ragwort is an extremely aggressive noxious weed that has spread in the Sheppard and Griffin Creek watershed areas burned during the 1994 Little Wolf forest fire. The purpose of this project is to prevent the tansy ragwort plants from going to seed and spreading the weed infestation.

Tansy ragwort is toxic to livestock (i.e., cattle, horses, and pigs). The plants contain alkaloids that cause liver damage. Tansy ragwort is also a non-native plant that can outcompete native vegetation, with each plant producing approximately 150,000 seeds. It is important to prevent the current infestation from going to seed. The Tally Lake Ranger District is preparing this EIS because Forest Service policy requires such documentation whenever aerial application of chemical pesticides is proposed.

The proposed action involves application of the herbicide clopyralid (0.25 lbs of active ingredient per acre) from a helicopter during the spring and fall of 1997 and 1998. No aerial spraying would occur within 100 feet of watercourses or on approximately nine acres of extremely wet ground. Hand spraying with 2,4, D amine (aquatic label), picloram (Tordon), and/or a mixture of 2,4 D and dicamba (Weedmaster) would be done within the 100 foot buffer zones along watercourses, in narrow strips between buffer zones, within spot infestation sites, and as needed after aerial spraying to kill tansy ragwort plants that may persist. Hand pulling of plants would occur immediately adjacent to streams and in extremely wet areas. In addition consideration will be given to use of biological control agents such as the cinnabar moth, tansy ragwort flea beetle, and the ragwort seed fly. Road management actions including road closures, spraying and weed pulling along roads, washing logging trucks, etc., would also be employed to prevent transport of seeds outside infested areas. Education efforts and intensive monitoring for tansy ragwort would also occur.

Alternatives to the proposed action include no action; a 2,4 D aerial spraying alternative; and a hand spraying alternative. The 2,4 D aerial spraying alternative would be the same as the proposed action except that 2,4, D would be used instead of clopyralid. The herbicide 2,4 D is less expensive than clopyralid, and would also be effective against tansy ragwort.

The herbicide 2,4 D, however, is not as selective as clopyralid and would kill more non-target plant species including conifer seedlings.

The hand spraying alternative would involve only use of ground based herbicide application methods (e.g., backpack tanks, backpack boom). Since less drift of herbicide is anticipated with hand spraying the buffer zones around streams would be reduced to 50 feet. This alternative reduces the potential for herbicides to drift into streams.

The transmittal letter with the DEIS, dated March 25, 1997, indicated that the proposed action is the District Ranger's preferred alternative at this time. This letter also indicates that for spring 1997 hand spraying of clopyralid rather than aerial spraying would be included in the preferred alternative since little time is available to prepare a helicopter spraying contract.

COMMENTS:

1. We commend the Forest Service for including the discussion of research results and experience with handpulling of tansy ragwort plants (pages 2-10, 11). This discussion evidences a proactive approach to disclosing and explaining to the public potential options for addressing the weed infestation, and explaining why they were dropped from further consideration.

2. If aerial spraying must be used to control the noxious weed spraying we support use of clopyralid for aerial spraying rather than 2,4 D since clopyralid would kill fewer non-target plants.

3. We recommend that the Procedures for Mixing, Loading and Disposal of Pesticides, a Spill Plan, and Aerial Spray Recommendations and Mitigation Measures be included in an appendix of the FEIS to assure that applicators and the public understand the safety measures and precautions to be used. Attached is an example of such procedures taken from Appendices of the Lolo National Forest's Mormon Ridge Winter Range Restoration FEIS, May 1996.

4. It is stated on page 1-13 that if sufficient vegetation is killed it may warrant revegetation efforts. We believe that revegetation (reseeding with native grass mix) should be expanded to seed any site within the control area where the vegetation density is low enough to allow reinfestation of tansy ragwort, introduction of other noxious weeds, or erosion. The goal of the seeding program should be to establish the sustainability of the area and should not be limited to the tansy ragwort problem.

5. We believe it would aid in understanding and review of the FEIS if an improved map(s) was included to more clearly display the locations of proposed herbicide application areas relative to locations of streams, springs, wetlands, and other surface waters.

6. We agree that if this tansy ragwort infestation is not controlled the infestation could spread and result in wider use of herbicides (DEIS page 3-21), which could correspondingly have adverse impacts on water quality and fisheries. However, this does not mean that adequate precautions should not be taken presently with the Tansy Ragwort Control Project to avoid delivery and transport of herbicides to surface and ground water as much as possible.

7. It is stated in the DEIS that no herbicide spraying would occur in standing surface water (page 3-10) or on nine acres of "extremely wet" ground (page 1-9). This proposed limitation to avoid spraying in "extremely wet" ground is not clear. We believe it should be unequivocally stated that no herbicide spraying will occur in wetlands. Wetlands are those areas that are inundated or saturated by surface or ground water at a frequency and duration sufficient to support and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions. Wetlands generally include swamps, marshes, fens and similar areas.

We recommend that wetlands in the area to be sprayed be identified and flagged on the ground to assure that herbicide applicators are aware of the location of wetlands, and thus, can avoid spraying in or near wetlands.

8. We recommend that all areas to be sprayed be flagged to minimize the area that is sprayed, prevent spray application to incorrect sites, and increase the safety for the pilot by eliminating the pilot's need to study contour maps while operating the helicopter. A person on the ground, at or close to the spray site, maintaining radio contact with the pilot, should also be included in the protocol. If the topography is very steep and mountainous, the helicopter may not be able to fly very close to the ground. Release of herbicides at higher altitudes will increase drift. Also, flagging will need to be visible from these altitudes. In the gypsy moth control program in the eastern U.S. they use brightly colored balloons to mark spray sites. Such flagging is recommended on all avoidance areas.

9. The discussion of wetlands on page 3-26 does not include adequate evaluation of the water quality and ecological impacts of herbicide drift into wetlands, particularly potential effects of herbicide contamination on wetland functions. For example, we would expect that herbicide drift into wetlands could adversely

affect wetland functions such as food chain support and habitat for wetland species. Such impacts should be evaluated and disclosed.

10 We are concerned about the potential for picloram and clopyralid to be transported to surface and ground waters. Clopyralid is closely related structurally to picloram (3, 6, Dichloropicolinic acid). The Montana Department of Agriculture (MDA) considers clopyralid to have a high potential for leachability, since it does not readily adsorb to soils, doesn't photo degrade, and doesn't volatilize. The MDA has found picloram and clopyralid in ground water in the Fairfield Bench area northwest of Great Falls where there are sandy clay soils. Clopyralid levels in ground water have been in the part per billion levels, below those considered a risk for human health.

It is stated on page 2-6 of the DEIS that picloram will only be used on sites away from streams and where the water table is deeper than six feet below the surface. We are concerned that six feet of depth to ground water may be inadequate to prevent leaching of picloram to ground water. The Montana Department of Agriculture considers 50 feet of soil depth to be sufficient depth of soil to mitigate the potential for the movement of picloram or clopyralid to ground water (Donna Rise, MDA, phone 444-5400), although less permeable soils may allow reduction in this safe soil depth to ground water.

There is inadequate information in the DEIS regarding soil characteristics and ground water levels to evaluate potential for herbicides to leach to ground water.

The discussion of "Movement of Herbicides in Soil Water" on page 3-16 of the DEIS does not adequately evaluate and disclose the potential of herbicides to leach into and contaminate ground water. Clopyralid has a water solubility of approximately 300,000 ppm, a relatively low adsorption coefficient, and a moderate half life (approximately 40 days). Potential for clopyralid (and picloram) to leach to ground water exists.

The description of soil characteristics in the DEIS indicates that landtype 26D has a 7 inch silt loam ash layer (2-6% organic), a 17 inch silt loam glacial till layer, and a 24 inch layer of compacted till. We have several comments and questions regarding the soils in the area to be sprayed.

a) Are these soil layers and characteristics consistent on the over 1,000 acres of infested area to be sprayed, including the 80 areas of spot weed infestations? If not, how do soil characteristics vary from site to site? We are most concerned if there are areas with highly permeable, sandy gravelly soil with high ground water.

b) The three soil layers on landtype 26D comprise a depth of 48 inches. What lies below the depth of 48 inches?

c) Mention is made of a perched water table and of saturated soil layers on page 3-16, however, we do not see depth to ground water or seasonal variation of ground water depth displayed. What is the depth to ground water and seasonal variation in ground water depth in the proposed areas to be sprayed? We suggest that the Forest Service contact the Ground Water Information Center at the Montana Bureau of Mines & Geology in Butte, MT at 496-4153 to see if there is well log information for the area that would help establish ground water levels.

11. The relationship of the discussion of clopyralid contamination of ground water at the Fairfield Bench (bottom of page 3-16) to the Tansy Ragwort Control Project is unclear. We suggest that the herbicide applications and site characteristics at these two locations be more clearly compared, and the potential for herbicide contamination in ground water more completely evaluated.

12. We did not see discussion in the DEIS of herbicide application relative to rainfall potential. The herbicide applicator should take the precaution of monitoring weather reports before spraying to assure application of herbicide only when there is minimal likelihood of rainfall within 24 hours of spraying.

13. It is stated on page 3-19 of the DEIS that the project will occur primarily in the Hand Creek watershed, and that Hand Creek flows approximately 14 miles before reaching Star Meadows where the water is used to water livestock. Is this Hand Creek water use 14 miles downstream from the herbicide application area the closest downstream agricultural use of water? Are there any potable water uses downstream or downgradient from any of the herbicide application areas?

We believe that all potable, agricultural, and recreational uses of surface and ground water immediately downstream or downgradient from proposed herbicide application areas should be clearly disclosed and evaluated for potential effects from herbicide applications.

14. We note in particular that picloram can persist and be transported in water systems for long periods (e.g. picloram solubility in water of 430 mg/l). Picloram is also relatively toxic to aquatic life having a 96 hour LC50 of 3.5 mg/l (cutthroat trout). We also note that Tordon (picloram) application by a County Weed District in Wyoming (in accordance with herbicide label restrictions) resulted in transport of picloram through ground water a distance of several miles.

Subsequent pumping of downstream ground water for household use resulted in the death of garden and household plants, evidencing the continuing presence of picloram in ground water.

Mr. Edward Stearns, pesticide specialist in EPA's Denver Regional Office (telephone number (303) 312-6946), can provide further information regarding this particular episode of ground water contamination from picloram application. We note that more permeable soils allow for transport and movement of contaminants in ground water.

15. While the EPA is supportive of the proposed project purpose we believe adequate resources should be obtained to allow some level of water quality monitoring to be conducted to measure actual herbicide impacts to surface and ground waters and the aquatic ecosystem. We believe the health of downstream domestic, agricultural and recreational water users and of the aquatic ecosystem should dictate some level of aquatics monitoring to document and verify that aqueous transport of picloram, clopyralid, 2,4 D and/or dicamba in significant amounts did not occur.

While it is stated (DEIS page 1-15) that water quality monitoring would occur in years of aerial application of herbicides, and that water quality monitoring would be done to determine if measurable amounts of herbicides entered streams (page 1-12), very little specific information regarding the water quality monitoring program is provided.

The EPA believes that water quality/aquatics monitoring is a necessary and crucial element in identifying and understanding the consequences of one's actions, and should be an integral part of any management decision. This monitoring program should be displayed in the FEIS to allow the adequacy of the monitoring program to be evaluated, and to assure that project effects on water quality (i.e., chemical and biological impacts) will be detected.

At a minimum, we believe that area streams should be sampled before the spraying, immediately following spray application, and immediately after the first major rainfall following application. The monitoring program should display sampling locations relative to area of herbicide treatment, parameters to be monitored, methodologies to be used, frequency, pattern and number of samples to be collected, etc.,. Without this information the DEIS is inadequate to fully assess the role of monitoring and evaluation in project implementation.

We are also enclosing information regarding a rapid bioassay technique that may be of assistance in detecting herbicides in water (see attached). While the attached paper (provided to us by Mr. Stearns) describes procedures for detection of herbicides other than picloram and clopyralid this leaf disc buoyancy procedure may have applicability to picloram and clopyralid.

We also note that bioassay techniques using aquatic species sensitive to the herbicides to be used would be appropriate for detecting aquatic impacts from herbicide applications (e.g., stoneflies, cuttroat trout). EPA has prepared a toxicity testing manual entitled, "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms", EPA/600/4-90/027, September 1991. Toxicity testing procedures are described in this manual, including procedures using rainbow and brook trout. If you have questions regarding toxicity testing procedures we encourage you to contact Mr. Glenn Rodriguez at our EPA Denver Office at (303) 312-6832, or Mr. Loys Parrish at EPA's laboratory at the Denver Federal Center at (303) 236-5055.

We encourage the Forest Service to consider conducting before-and-after bioassays in surface and ground waters in the potentially affected Hand Creek drainage. Actual impacts to the aquatic ecosystem and public health from proposed herbicide applications can only be detected through monitoring.

16. The statement on page 3-38 (also on page 2-18) of the DEIS indicating that all four herbicides to be used have undergone extensive testing for cancer, and that clopyralid, picloram and dicamba are classed by EPA as non-carcinogenic is somewhat misleading. Evaluation of the carcinogenicity of these chemicals is an ongoing process, and as studies progress, information may change.

From the studies to date, it does appear that picloram can be considered non-carcinogenic. Clopyralid has not evidenced carcinogenicity in studies, however, formal classification as non-carcinogenic has not been conducted during EPA Peer Review. In regard to dicamba, the EPA Peer Review Committee determined that the doses selected for rat and mouse studies were not adequate, dicamba, therefore, remains non-classifiable as to human carcinogenicity. The classification of the carcinogenicity of 2,4, D is pending repeat of studies and additional epidemiological data. The herbicide 2,4 D remains non-classifiable as to human carcinogenicity.

We also believe that health concerns other than carcinogenicity stemming from possible exposure to low levels of herbicides, such as endocrine disruption or reproductive effects should be addressed in the FEIS. There is controversy over possible endocrine effects of 2, 4, D.

17. For public disclosure purposes we recommend that the table showing the acute toxicity levels of the proposed four herbicides to be used (clopyralid, 2,4, D amine, picloram and dicamba), that is referenced on page 3-22 of the DEIS, be displayed in the FEIS.

18. To better meet the public disclosure purposes of NEPA we also recommend that the pesticide labels showing the use precautions and restrictions for herbicides to used (i.e., clopyralid, 2,4 D, picloram, and dicamba) be shown in the appendices of the FEIS.

ENCLOSURE

From Mormon Ridge Winter Range Restoration FEIS

May 1996

# APPENDIX A

# PROCEDURES FOR MIXING, LOADING, AND DISPOSAL OF PESTICIDES

The following measures will apply to all pesticide applications.

1. All mixing of pesticides will occur at least 100 feet from surface waters or well heads.

2. Dilution water will be added to the spray container prior to addition of the spray concentrate.

3. All hoses used to add dilution water to spray containers will be equipped with a device to prevent back-siphoning.

4. Applicators will mix only those quantities of pesticides that can be reasonably used in a day.

5. During mixing, mixers will wear a hard hat, goggles or face shield, rubber gloves, rubber boots, and protective overalls.

6. All empty containers will be triple rinsed and rinsate disposed of by spraying near the application site at rates that do not exceed those on the spray site.

7. All unused pesticide will be stored in a locked building in accord with pesticide storage regulations contained in Forest Service Handbook 2109.14.

8. All empty and rinsed pesticide containers will be punctured and disposed of in a sanitary landfill.

In addition the section from the *Northern Region Emergency and Disaster Plan* entitled "Hazardous Materials Releases and Oil Spills" will be reviewed with all appropriate personnel (see following pages). Notification and reporting requirements as outlined in this section will be followed in the unlikely event of a serious spill.

## HAZARDOUS MATERIALS RELEASES AND OIL SPILLS

(Excerpted from the *Northern Region Emergency and Disaster Plan*)

**AUTHORITY:** Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA); and Superfund Amendments and Reauthorization Act of 1986 (SARA). Other statutes that may apply include Resource Conservation and Recovery Act (RCRA); Hazardous and Solid Waste Amendments (HSWA); Toxic Substances Control Act (TSCA); Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Clean Water Act (CWA); and Clean Air Act (CAA).

**DEFINITION:** A hazardous materials emergency or oil spill is defined as any release or threat of release of a hazardous substance or petroleum product that presents an imminent and substantial risk of injury to health or the environment.

A release is defined as any spilling, leaking, pumping, pouring, emitting, emptying, discharging, injecting, escaping, leaching, dumping, or disposing into the environment.

Releases that do not constitute an immediate threat, occur entirely within the work place, are federally permitted, or are a routine pesticide application, are not considered to be an emergency and are not covered by this direction.

**RESPONSIBILITY:** The first person who knows of a release and is capable of appreciating the significance of that release has the responsibility to report the release.

Only emergency release response and reporting is covered by this direction. Non-emergency reporting will be accomplished by appropriate RO staff specialists who should be notified directly of all non-emergency releases.

An emergency release of a hazardous substance or petroleum product may be from a Forest Service operation or facility; from an operation on National Forest land by a permit holder, contractor, or other third party; or from a transportation related vehicle, boat, pipeline, aircraft, etc., crossing over, on, or under Forest lands. Response and/or reporting by Forest Service employees will differ in each situation:

1. If the release is from a Forest Service facility or operation, the Forest Service and its employee(s) is clearly the "person in charge," and is fully responsible for all reporting. Immediate response action is limited to that outlined in emergency plans and only to the extent that personal safety is not threatened.

2. If the release is from a third party operation, the Forest Service will only respond and/or report the emergency if the third party fails to take appropriate action.

3. If the release is from a transportation related incident, the Forest Service will only respond and/or report the emergency if the driver or other responsible party is unable or fails to take appropriate action.

# HAZARDOUS MATERIALS RELEASES AND OIL SPILLS -- CONTACT LIST AND IMMEDIATE ACTION GUIDE

**ORGANIZATIONS FOR EMERGENCY AND TECHNICAL ASSISTANCE:**

**Consult the Northern Region Emergency and Disaster Plan for a list of contacts in the event of a spill.**

**Consult the Northern Region Emergency and Disaster Plan for a list of contacts and response actions in the event of a spill.**

# APPENDIX C

# AERIAL SPRAY RECOMMENDATIONS AND MITIGATION MEASURES

The following recommendations were provided by Jack Barry, Director, Forest Health Technology Enterprise Team, USDA Forest Service, Davis, California.

1. The treatment block should be marked with flagging extended on trees or poles to mark the block corners. It would also be desirable to mark the swath lanes at 100 foot intervals at each end with flagging, and to use a flagger at each end who would move up slope with each succeeding swath. This procedure would add to project costs but would nearly ensure an even application. Experience has clearly demonstrated (results published), that show pilots do not fly parallel, even-spaced swaths without assistance, in complex terrain. I do not believe that Global Positioning Satellite (GPS) guidance in real-time is suited for this type project at this time. However, it would be desirable to have a GPS system on board to record helicopters swaths, position, and boom on and off times and location.

2. Winds in the canyon should follow the typical diurnal pattern of upslope during the day and downslope during the night. I refer to canyon winds as those that move up or down the canyon drained by Mormon Creek; and slope winds as those that move up or down the slope immediately south of Mormon Peak. These diurnal winds result form heating and cooling of the surface. Clear skies with solar radiation reaching the surface during the day, cause up canyon and upslope winds. Cooling that occurs after sunset, generates upslope or drainage winds. It will be essential to avoid drift down canyon (west to east) and downslope (north to south) into Mormon Creek, a perennial stream that borders the treatment block on the south. Down canyon and downslope winds will likely occur on clear days following daytime hours. It is therefore essential to spray when winds are up canyon and upslope, thus preventing spray from drifting down canyon (west to east). We can avoid drift into Mormon Creek and drift down canyon by taking several steps as follows:

a. Spray in the morning when up canyon and upslope winds are well established and blowing up canyon and away from Mormon Creek. The specific time to be determined by real-time weather monitoring.

b. Apply spray at 5 gallons per acre in large drops using the D-8 jet nozzle (no whirl plate) oriented straight back. Wind tunnel tests show that this nozzle, used as we intend, produces a volume median droplet (vmd) of 1246 micrometers. Restrict nozzle locations to no more than 75% of the helicopter's rotor length. Maintain boom pressure at 30 psi. Monitor spray pressure because the pilot may change pressure during flight. This changes application rates and may change drop size.

c. Flow rate calibration in gallons per minute = application rate of 5 gpa x 50 mph helicopter speed x 50 foot swath *divided* by 495 = 25.25. To determine the number of nozzles divide 25.25 by the flowrate of a single D-8 nozzle when operated at 30 psi, which is 1.62 gpm; therefore 25.25 *divided* by 1.62 = 15.19. Use 16 nozzles. I suggest the Forest Service provide the contractor with new nozzle and check valve systems, and that the Forest Service check helicopter calibration and characterize the spray system by having the helicopter fly over spray deposit cards.

d. Begin first swath 100 feet or more above Mormon Creek. This is the buffer/safety zone.

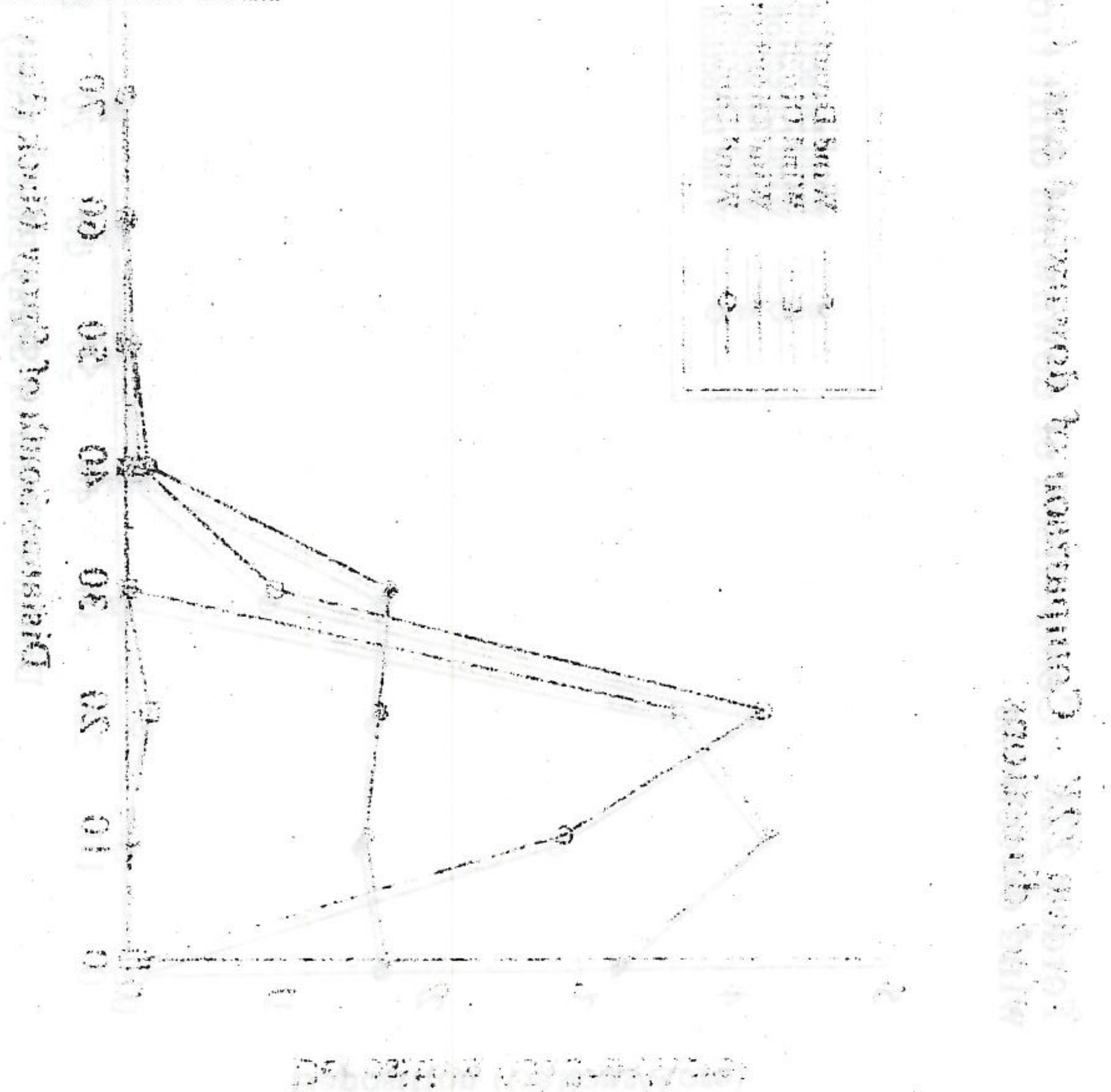
e. Clearly mark block boundaries so they are clearly visible to the pilot. Use human flaggers at end of each swath. Of course the flaggers will have to wear protective clothing.

Results of aerial spray drift modeling efforts are shown in part the accompanying figure. The entire modeling report is contained in the project file. Modeling runs clearly demonstrate that:

- Most of the spray is deposited in the treatment block regardless of wind direction;

- Direction of off-target deposition can be managed by monitoring the winds and conducting spray under conditions that will carry the spray away from the stream and;

- Even when spraying under wind directions of 45, 90, 240, and 300 degrees there is essentially no deposition in the stream with a buffer of 100 feet. Southerly winds as specified by this project (180 degrees, not shown here) would blow the spray upslope and away from the stream and ensure no drift contamination of the stream.



ENCLOSURE

Pestic. Sci. 1985, 16, 457–462

# A Rapid Bioassay for the Detection of Photosynthesis Inhibitors in Water

Sarina Saltzman and Bruria Heuer

*Institute of Soils and Water, Agricultural Research Organisation, The Volcani Center, Bet Dagan, Israel*

*(Manuscript received 3 January 1985)*

A modified leaf disc buoyancy procedure for the detection of photosynthesis-inhibiting residues in water is described. The modifications proposed, mainly the presence of sodium hydrogen carbonate in the infiltration solution, increased the sensitivity of the method and reduced the time required. The substituted urea and 1,3,5-triazine herbicides diuron, linuron, monuron, atrazine, ametryn and atraton were detected below 0.7 mg litre$^{-1}$ using cucumber (*Cucumis sativus* L., cv. 'Dalia') leaf discs. A concentration as low as 0.09 mg diuron litre$^{-1}$ could be detected. Although bean (*Phaseolus vulgaris* L., cv. 'Bulgarian') leaf tissue was less sensitive in this bioassay than cucumber, 0.3 mg diuron litre$^{-1}$ could still be detected. The test, being very rapid (less than 30 min per determination) and relatively sensitive, could be used for the detection of photosynthesis inhibitors in recycled water used for irrigation.

## 1. Introduction

The use of waste water for irrigation in arid and semi-arid regions is an attractive disposal technique from both the economic and ecological points of view. Treating effluents to a high quality standard is usually costly and requires advanced technology: therefore waste water which has undergone varying levels of treatment is frequently used for irrigation. Residual organic compounds not removed by conventional sewage treatments could be phytotoxic to crops. Some of these chemicals are photosynthesis inhibitors. Assessment of the phytotoxicity of a complex, ill-defined solution can be done only by biological tests.

The bioassay techniques commonly used for the detection of photosynthesis inhibitors in a medium are based on the response of plants grown in the medium. The main drawbacks of many such tests are that they are time-consuming and non-specific and often include the destruction of the test plants. Specific bioassay techniques for photosynthesis inhibitors which have been developed include the use of green algae,[1-3] the inhibition of specific reactions of isolated chloroplasts,[1] the measurement of leaf chlorophyll fluorescence,[4] and the leaf-disc buoyancy method.[5-7] As the last technique is simple, rapid, and does not require sophisticated equipment, its adaptation for the detection of photosynthesis inhibitors in recycled water was considered.

The leaf disc buoyancy (LDB) procedures for the detection of photosynthesis inhibitors are based on the ability of photosynthesising leaf discs to float, apparently because of the high oxygen concentration in the leaf tissues. Blocking photosynthesis, either by lack of light, or by the presence of a photosynthesis inhibitor, stops oxygen production and induces disc sinking. The first LDB procedure developed, worked with cotyledon leaf discs only, and required 12–24 h per test.

A later modification of this procedure used infiltration of the leaf discs by the test solutions under vacuum until buoyancy was lost. The sunken discs were exposed to light in a similar medium to that used for infiltration, to which sodium hydrogen carbonate had been added, and the time taken for the discs to float was determined.[8] This procedure, which worked with true leaves, was used for the identification of triazine-resistant and -susceptible weeds.

The purpose of the present work was to adapt the basic LDB test, as modified by Hensley,[8] for the rapid detection of photosynthesis inhibitors in recycled water, using cultivated plants as the test species.

## 2. Experimental methods

Cucumber plants (*Cucumis sativus* L., cv. 'Dalia') were grown in a greenhouse in continuously aerated half-strength Hoagland solution. Bean plants (*Phaseolus vulgaris* L., cv. 'Bulgarian') were also grown in aerated Hoagland solution in a growth chamber at 25 ($\pm$1)°C and 50 ($\pm$5)% relative humidity. A light intensity of 18 000 lux (350 $\mu$E m$^{-2}$ s$^{-1}$) at the plant tops was provided by fluorescent lamps for 13 h per day. Deionised water was added regularly to the culture solutions to replace losses due to transpiration.

Chromatographically pure (>99%) standards of the herbicides diuron, linuron, monuron, atrazine, ametryn and atraton were used in all studies. The recycled water samples were secondary treated effluents of domestic origin from two municipal treatment plants (Qiryat Shmona and Hertzliya) and from an oxidation pond near Nazareth (Tel-Adashim). The characteristics of these effluents, which were used for crop irrigation, were within the limits of conventionally treated secondary effluents [suspended solids 20–40 mg litre$^{-1}$, biological oxygen demand (BOD) 15–35 mg litre$^{-1}$, chemical oxygen demand (COD) 30–70 mg litre$^{-1}$, ammonia-N 15–35 mg litre$^{-1}$, phosphorus-P 6–12 mg litre$^{-1}$]. In addition, a raw sewage sample (suspended solids 299, BOD 384, COD 599 mg litre$^{-1}$) from Hertzliya was tested.

Discs (9 mm diam.) were cut from fully expanded, young leaves of both species and transferred to 0.01-M potassium phosphate buffer, pH 7.5, containing 0.1-M sodium hydrogen carbonate, or to the same solution containing a variable concentration of herbicide, or to the sewage effluent samples, containing 0.1-M sodium hydrogen carbonate. Cucumber plants were 6 weeks old and bean plants were 3 weeks old when the leaves were harvested for the experiments. Leaves of the same age and taken from the same position on the plants (second fully expanded leaf) were used in all experiments. The discs were infiltrated under vacuum (25 mmHg) for 5 min (sinking time) in 250-ml Erlenmeyer flasks containing the above solutions. The flask contents were then transferred to 100-ml beakers and kept in the dark for 5 min. The beakers were then exposed to a light source provided by a Philips HPLR, 250-W lamp (350 $\mu$E m$^{-2}$ s$^{-1}$).

All the treatments were replicated at least four times, each replicate consisting of 20 discs. Each experimental set included a control treatment to which no photosynthesis inhibitor was added. The time required for all the discs to float to the surface of the medium was recorded, and the results were expressed as the ratio between the refloating time in the test solutions and that in the control for each set; this ratio is the retardation index (RI). The higher the RI, the stronger was the inhibition of photosynthesis. Means of all the data were used for calculation of the RI.

## 3. Results

Leaf discs of both bean and cucumber that were infiltrated under vacuum with 0.01-M potassium phosphate buffer, in the presence of sodium hydrogen carbonate, sank in a few minutes, but resurfaced upon exposure to light. Preliminary experiments showed that the time required for the refloating of bean discs varied within narrow limits (3–7 min), and was affected by the experimental conditions (Table 1). Increasing the infiltration time above 5 min seemed to reduce the time required for resurfacing while the increased time in darkness delayed the refloating of the discs. The time in darkness did not appear to affect the refloating time at a concentration of 12.5-mM sodium hydrogen carbonate (Table 1). The same trend was observed with cucumber leaf discs.

As a preliminary experiment (data not presented) had shown that the immediate transfer of the infiltrated discs to light resulted in a very fast resurfacing, it was necessary to increase the contact time with the herbicides by keeping the samples in darkness. A tendency for an increased refloating time after a longer period in darkness was observed, mainly at the higher concentration of sodium hydrogen carbonate.

Table 1. The relationship between the infiltration time, the dark period, and the refloating time for bean discs

| Infiltration time (min) | Time in darkness (min) | Refloating time[a] (min) 100-mM $HCO_3^-$ | 12.5-mM $HCO_3^-$ |
|---|---|---|---|
| 5 | 5 | 3.68 (±0.10) | 5.60 (±0.22) |
| | 10 | 3.82 (±0.15) | 6.50 (±0.25) |
| | 15 | 5.15 (±0.22) | 6.66 (±0.25) |
| 10 | 5 | 3.90 (±0.10) | 5.43 (±0.20) |
| | 10 | 4.25 (±0.20) | 5.96 (±0.15) |
| | 15 | 5.03 (±0.15) | 5.46 (±0.17) |
| 15 | 5 | 3.33 (±0.30) | 5.03 (±0.15) |
| | 10 | 3.48 (±0.15) | 5.61 (±0.20) |
| | 15 | 4.43 (±0.18) | 5.51 (±0.20) |

[a]Data are the means of four replications with 20 discs per replication.

The need for the hydrogen carbonate ion in the incubation medium was tested over the concentration range of 0–100 mM (Figure 1). In order to ensure that sufficient hydrogen carbonate would be present in the leaf discs at the time of light exposure, and to prevent the anticipated lag period before maximal photosynthesis rate was reached, the hydrogen carbonate must also be present during the infiltration period. With discs of bean leaves, a very strong inhibition of the rate of photosynthesis was observed over the range of 0–12.5-mM sodium hydrogen carbonate; at concentrations higher than 25 mM, the maximal rate was obtained. Although cucumber leaf discs responded well in the presence of 100-mM sodium hydrogen carbonate, a very strong photosynthesis inhibition was observed at 50 mM. Therefore, in order to obtain uniform experimental results, 100-mM sodium hydrogen carbonate was used in all the experiments.

The inhibitory effects of three herbicides from the substituted-urea group on the leaf disc photosynthesis are shown in Table 2. While linuron and monuron behaved in a similar manner,

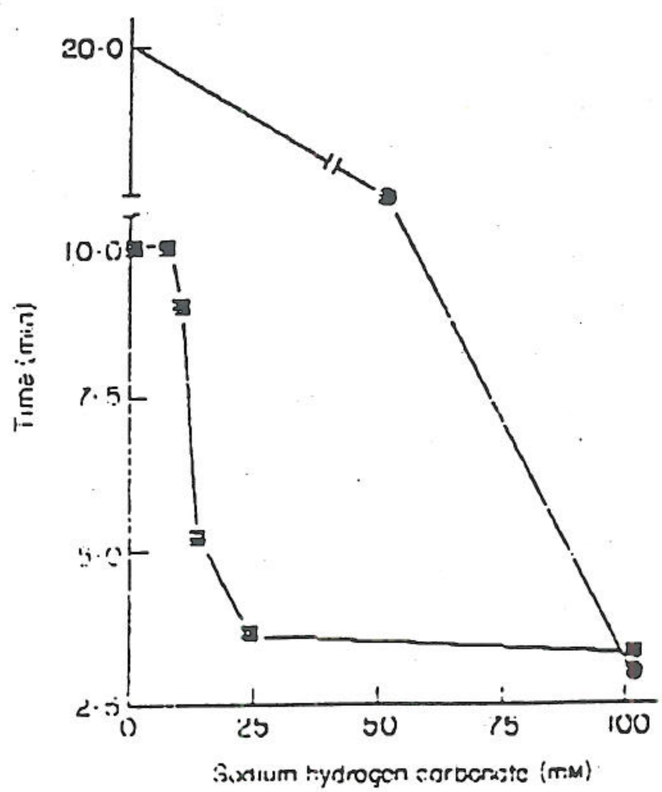



Figure 1. The effect of the sodium hydrogen carbonate concentration in the incubation solution on the refloating time of: (■) bean leaf discs, (●) cucumber leaf discs. Both the infiltration time and the time of darkness were 5 min.

Table 2. The effect of substituted urea herbicides on the retardation index (RI) of cucumber and bean leaf discs

| Herbicide | Herbicide concentration (mg litre$^{-1}$) | Retardation index[a]: Cucumber leaf discs | Retardation index[a]: Bean leaf discs |
|---|---|---|---|
| Diuron | 0.09 | 1.22 (±0.02) | NI |
| | 0.30 | 1.25 (±0.08) | 1.03 (±0.13) |
| | 0.50 | 1.70 (±0.10) | 1.06 (±0.14) |
| | 0.70 | 3.18 (±0.28) | 1.65 (±0.02) |
| | 1.00 | 4.08 (±0.23) | 2.09 (±0.18) |
| Linuron | 0.30 | NI | NI |
| | 0.50 | 1.27 (±0.08) | NI |
| | 0.70 | 2.22 (±0.20) | NI |
| | 1.00 | 3.27 (±0.17) | 1.55 (±0.20) |
| | 1.50 | — | 2.55 (±0.08) |
| Monuron | 0.30 | NI | NI |
| | 0.50 | 1.16 (±0.06) | NI |
| | 0.70 | 2.27 (±0.18) | NI |
| | 1.00 | 3.25 (±0.15) | 1.19 (±0.17) |
| | 1.50 | — | 2.55 (±0.07) |

[a]Retardation index = (refloating time in medium + herbicide)/(refloating time in medium without herbicide).

NI—no inhibition (RI<1).

diuron showed a much stronger inhibition, as demonstrated by the higher RI values. Diuron concentration as low as 0.09 mg litre$^{-1}$ reduced the LDB of cucumber by 22%, while for bean leaf discs at least 0.3 mg litre$^{-1}$ was needed for even a slight inhibition. Generally, the sensitivity of cucumber was much greater than that of bean. At a concentration of 0.7 mg diuron litre$^{-1}$, the refloating time was increased more than three-fold with cucumber, while only 65% increase was observed with bean leaf discs. Linuron and monuron inhibited the photosynthesis of cucumber leaf discs only at concentrations of 0.5 mg litre$^{-1}$ or greater, while with beans the minimum concentration needed to produce an effect was 1 mg litre$^{-1}$.

Herbicides from the 1,3,5-triazine group were also treated for their inhibitory effect on the LDB of cucumber and bean leaf discs. The results (Table 3) showed that at the lowest concentrations tested, atraton and ametryn inhibited the photosynthesis of cucumber discs, while atrazine had no effect. As with the substituted urea herbicides 0.7 mg litre$^{-1}$ was the minimum concentration required to cause severe inhibition (RI more than 2) of the photosynthesis of cucumber leaf discs. Bean leaf discs were less sensitive to 1,3,5-triazines than cucumber, the minimum concentrations affecting photosynthesis being 0.7 mg litre$^{-1}$ for atraton, and 1 mg litre$^{-1}$ for atrazine or ametryn. Using bean leaf discs, the minimum concentration necessary to induce severe inhibition was 1.5 mg litre$^{-1}$ for all the 1,3,5-triazine herbicides tested.

Several experiments were carried out using discs cut from leaves of cucumber plants grown in a growth chamber under the conditions described for the growth of the bean plants. These discs seemed to be less sensitive to photosynthesis inhibitors than those cut from plants grown in the greenhouse (data not presented). This difference was reflected more in the magnitude of the response than in the minimum herbicide concentration required for inhibition.

Table 3. The effect of 1,3,5 triazine herbicides on the retardation index (RI) of cucumber and bean leaf discs

| Herbicide | Herbicide concentration (mg litre$^{-1}$) | Retardation index*: Cucumber leaf discs | Retardation index*: Bean leaf discs |
|---|---|---|---|
| Atrazine | 0.5 | NI | NI |
| | 0.7 | 2.41 (±0.28) | NI |
| | 1.0 | 2.50 (±0.21) | 1.07 (±0.02) |
| | 1.5 | — | 2.03 (±0.13) |
| Ametryn | 0.3 | 1.29 (±0.05) | NI |
| | 0.5 | 1.48 (±0.04) | NI |
| | 0.7 | 2.82 (±0.15) | — |
| | 1.0 | 4.46 (±0.32) | 1.73 (±0.11) |
| | 1.5 | — | 2.59 (±0.25) |
| Atraton | 0.3 | 1.20 (±0.03) | NI |
| | 0.5 | 1.54 (±0.09) | NI |
| | 0.7 | 3.17 (±0.41) | 1.10 (±0.10) |
| | 1.0 | 3.32 (±0.31) | 1.09 (±0.06) |
| | 1.5 | — | 2.40 (±0.16) |

*Retardation index=(refloating time in medium+herbicide)/(refloating time in medium without herbicide).

NI - no inhibition (RI≯1).

Table 4. The effect of wastewater on the refloating time of cucumber leaf discs

| Sample | Refloating time (min): No inhibitor added | Refloating time (min): Diuron added (1 mg litre$^{-1}$) |
|---|---|---|
| Buffer solution | 2.32 (±0.03) | >10 |
| Tap water | [illegible] | >10 |
| Treated effluent (Tel-Adashim) | 2.18 ([illegible]) | >10 |
| Treated effluent (Qiryat Shmona) | 2.55 (±0.06) | >10 |
| Treated effluent (Hertzliya pond A) | 2.48 (±0.11) | >10 |
| Treated effluent (Hertzliya pond B) | [illegible] | >10 |
| Raw waste water (Hertzliya) | 7.47 (±0.50) | >10 |

The suitability of the modified LDB test for the detection of photosynthesis inhibitors in recycled water was also tested. Secondary treated sewage effluents used for irrigation did not affect the refloating time of cucumber leaf discs (Table 4). However, the raw sewage sample tested caused severe photosynthesis inhibition (RI 3.22). In order to determine whether the soluble and suspended components of the sewage effluents interfered with the determination of the presence of photosynthesis inhibitors by the LDB technique, the effluent samples were fortified with 1 mg diuron litre$^{-1}$. The results obtained (Table 4) show that the sensitivity of cucumber leaf discs to diuron present in sewage effluent samples was similar to that in tap water or buffer solution.

## 4. Discussion

The present work was aimed to adapt the LDB technique for the detection of photosynthesis inhibiting compounds in recycled water that was to be used for irrigation. Such a procedure must be fast and versatile, sensitive to different known photosynthesis inhibitors, work with cultivated plants, and in experimental conditions close to natural ones. This required several alterations of the existing technique. One of them is the presence of sodium hydrogen carbonate in the infiltration solution, in addition to the medium used for exposure to light. The role of the sodium hydrogen carbonate is to provide carbon dioxide for the photosynthesis process. Carbon dioxide is lost during the infiltration of leaf discs under vacuum, thus delaying the start of photosynthesis after exposure to light. Infiltrating the leaf discs with solutions containing sodium hydrogen carbonate eliminates the lag period caused by the loss of carbon dioxide, so that photosynthesis can start immediately after exposure to light. Consequently, the experimental time decreased; only 3–7 min were needed for all the sunken discs to refloat, following exposure to light in check solutions.

The dark period, the additional step introduced in the experimental procedure between infiltration and exposure to light, delays photosynthesis (an increase of this period from 5 to 15 min increased the refloating time by 30–40%). Hence the most convenient refloating time in check solutions, can be chosen at will. In the present procedure, the experimental dark period was 10 min.

In order to mimic the normal growth conditions of plants, the experiments were carried out at high light intensities, similar to natural light. For the same reason, true leaf tissue from cultivated plants was used in this procedure. The sensitivity of the method was checked for six herbicides, which are known photosynthesis inhibitors. The use of cucumber leaf discs allowed for the detection of each of these herbicides in concentrations of 0.7 mg litre$^{-1}$.

As expected, the sewage effluents tested, which were successfully used for irrigation, did not inhibit photosynthesis in cucumber leaf discs. Moreover, an added photosynthesis inhibitor was detected by the LDB test without interference from the suspended and soluble components of the effluents (Table 4), thus indicating that this simple and rapid test should be suitable for field conditions.

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